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Detection of Enteroviral RNA in End-Stage Dilated Cardiomyopathy in Children and Adolescents

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Medical records and archival myocardial specimens of 33 children and adolescents with end-stage idiopathic dilated cardiomyopathy (IDCM) were collected to evaluate retrospectively the potential role of enteroviral persistence in the pathogenesis of IDCM. The clinical history and laboratory assessment of each patient were reviewed carefully in order to obtain information on the nature and etiology of infections in the past and at the time of diagnosis of cardiomyopathy. Sixty-four formaldehyde-fixed, paraffin-embedded myocardial specimens, obtained from endomyocardial biopsies (n = 5), explanted hearts (n = 10), or autopsies (n = 49), were studied by the polymerase chain reaction (PCR) and by in situ hybridization to detect enteroviral RNA in the specimens. Control specimens included 34 formaldehyde-fixed, paraffin-embedded myocardial specimens from children with other cardiomyopathies, metabolic diseases, structural heart defects, or various noncardiac malignancies. The presence of cellular RNA in the specimens was confirmed by amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA or β-actin mRNA as positive controls. Only one specimen from the 32 IDCM patients with appropriate myocardial specimens was positive for enteroviral RNA by PCR. Sequence analysis of the amplified viral segment showed a significant degree of homology between the viral sequence and echovirus 1. One specimen from the control patients also appeared positive by PCR, but sequence analysis of the amplified viral segment revealed it as rhinovirus 16. The results do not indicate any significant role for enteroviral persistence in end-stage childhood IDCM, although they need to be confirmed using a prospective study with fresh frozen specimens. However, mechanisms other than viral persistence may be more important in the progression of IDCM to end-stage heart failure in this age group. ***J. Med. Virol. 56:364–371, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: idiopathic dilated cardiomyopathy; infections; polymerase chain reaction

INTRODUCTION

Dilated cardiomyopathy is a heterogeneous group of myocardial diseases characterized by ventricular dilatation and impaired contractility [Richardson et al., 1996]. A link between myocarditis, commonly caused by enteroviruses, particularly coxsackie B viruses (CBVs) [Woodruff, 1980], and idiopathic dilated cardiomyopathy (IDCM) was suggested originally by the long-term outcome of some patients with acute myocarditis [Billingham and Tazelaar, 1986; Quigley et al., 1987] and by showing enteroviral antibodies in patients with chronic myocarditis and IDCM more often than in controls [Banatvala, 1983]. However, in the development of myocardial injury, viruses were considered mainly as triggers of autoimmune reactions. This was not only because abnormalities in both cell-mediated and humoral immunity were found in patients with IDCM [Anderson et al., 1982; Eckstein et al., 1982; Limas et al., 1989; Ansari et al., 1991; Caforio et al., 1992] but also because viruses could not be detected in myocardial specimens of the patients by conventional methods. Recent advances in molecular biological techniques have allowed very small amounts of viral RNA, even in replication-defective form, to be de-

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tected [Kandolf et al., 1987; Jin et al., 1990; Severini et al., 1993]. Earlier studies have shown enteroviral RNA by different hybridization methods [Kandolf et al., 1987; Archard et al., 1988; Bowles et al., 1989] or by the polymerase chain reaction [PCR; Jin et al., 1990; Weiss et al., 1991; Grasso et al., 1992; Keeling et al., 1992; Severini et al., 1993; Andreoletti et al., 1995, 1996] in up to 60% of the studied myocardial specimens from adult patients with IDCM. However, none of the previous studies has investigated specifically the persistence of enteroviral RNA in end-stage IDCM in the young.

A retrospective analysis was carried out of formaldehyde-fixed, paraffin-embedded myocardial specimens from 33 children and adolescents with IDCM to detect enteroviral genomic material in the specimens by PCR [Halonen et al., 1995; Arola et al., 1996] and by in situ hybridization [Kallajoki et al., 1990; Hohenadl et al., 1991]. Medical records of the patients were also reviewed for clinical history and laboratory assessment in order to obtain information on infections as well as signs of myocardial injury in the past and at the time of presentation.

MATERIALS AND METHODS

Patients and Specimens

Medical records of birth to 20-year-old children and adolescents with IDCM in 1980–1991 were collected retrospectively from the 5 university and 16 central hospitals in Finland [Arola et al., 1997]. The echocardiographic inclusion criteria for IDCM consisted of left ventricular end-diastolic dimension $\geqslant$2 standard deviations (SD) above normal according to the age and body surface area of the patient [Roge et al., 1978] and fractional shortening (FS) $\leqslant$26%. Patients with significant hemodynamic structural heart defects, valvular heart diseases, and heart diseases resulting from primary arrhythmias were excluded as were those with renal, endocrine, metabolic, and collagen vascular disorders with potential secondary myocardial involvement. Patients with a history of transient heart failure during acute infection were also excluded. Of 62 patients with IDCM, myocardial specimens were available from 33 (53%); these 33 patients comprised the patient material of the present study.

The patients' age varied from 1 day to 20 years (median 2.0 years); 20 (61%) of the patients were males. Medical records were reviewed to assess clinical history, with special emphasis on the nature and etiology of past infections as well as on the symptoms and signs of infection at the time of presentation. The data of each patient on the concentration of creatine kinase (CK), lactate dehydrogenase (LDH), and corresponding heart-specific isoenzymes (CK-MB, LDH-1) as indicators of myocardial injury were collected. Results of virus isolations and antigen detections, bacterial cultures, and serological tests were reviewed. Data on HLA typing available for eight patients assessed for heart transplantation were collected.

A total of 64 myocardial specimens from right ventricular endomyocardial biopsies (EMBs) (n = 5), explanted hearts after transplantation (n = 10), or autopsies (n = 49) were investigated. Two or more (up to five) samples were available from 15 patients. Serial samples (a sample from EMB followed by one or several samples from heart transplantation or autopsy) were obtained from five patients. The time from the diagnosis of IDCM to myocardial specimen collection ranged from 1 day to 10 years (median 5 months). Twelve of the children (36%) had histologic evidence of endocardial fibroelastosis. Myocarditis according to the Dallas criteria [Aretz et al., 1986] was excluded in all patients. The myocardial specimens were fixed originally in formaldehyde and paraffin-embedded for routine histopathology. They had been stored for 9 months to 15 years before the present examination.

Control Specimens

Myocardial specimens from 34 children (median age 3.2 years, range 1 day to 18 years; male/female ratio 1.6), including 11 patients with other forms of cardiomyopathy, 4 with inborn errors of metabolism and secondary myocardial involvement, 11 with structural heart defects, and 8 with various noncardiac malignancies, were studied. Four of the control specimens were obtained from explanted hearts at transplantation and two from pieces of the interventricular septum excised during septal myectomy for hypertrophic cardiomyopathy; all other samples were from autopsy.

Template Preparation for PCR

Five μm-sections from each paraffin-embedded specimen were cut using sterile techniques and placed in an Eppendorf-tube. The sections were deparaffinized with two 5-min washes in xylene followed by two rinses in absolute ethanol. Total nucleic acids were isolated by proteinase K-SDS digestion (at concentrations of 500 μg/ml and 0.5%, respectively) at 37° C overnight (at least 18 hr) followed by phenol-chloroform extraction and ethanol precipitation. The final pellet was dissolved in nuclease-free water, incubated at 56° C for 15 min, and stored at −70° C.

Reverse Transcription (RT) and PCR

First-strand cDNA was generated from 5 μl of extracted total nucleic acids (after heating them at 94° C for 10 min) in a 40 μl mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM dNTPs (Pharmacia, Uppsala, Sweden), 50 pmol primer (−) (Table I), 4 U RNAse inhibitor (Promega Corp., Madison, WI), and 20 U Moloney murine leukemia virus reverse transcriptase (Promega). The mixture was incubated at 37° C for 60 min.

Ten microliters of the cDNA mixture was added to tubes to yield a 100 μl reaction mixture containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.5 mM dNTPs, 50 pmol primers (−) and (+) (Table I), capable of detecting a wide range of picornaviruses [Halonen et al., 1995], and 1 U of thermostable DNA polymerase (Dynazyme™; Finnzymes,

TABLE I. Oligonucleotide Primers and Probe used for RT-PCR in the Present Study

Primer/Probe	Sequence	Amplicon length (bp)
Picornavirus[a]		
Primer (–)	5'-GAAACACGGACACCCAAAGTA-3'	
Primer (+)	5'-TCCTCCGGCCCCTGAATG-3'	120
Probe	5'-CCAAAGTAGTCGGTTCCGC-3'	
GAPDH[b]		
Primer (–)	5'-GCTCCTGGAAGATGGTGATGG-3'	
Primer (+)	5'-AGGTGAAGGTCGGAGTCAACG-3'	234
B-actin[c]		
Primer (–)	5'-CACTGTGTTGGCGTACAGGT-3'	
Primer (+)	5'-TCATCACCATTGGCAATGAG-3'	150

[a]Data from Halonen et al., [1995].
[b]Data from Hilton et al., [1993].
[c]Data from Ben-Ezra et al., [1991].

Espoo, Finland). The final mixture was overlaid with mineral oil and amplified using a Perkin-Elmer Cetus (Norwalk, CT) thermocycler. The tubes were first incubated at 94° C for 5 min, followed by 30 cycles at 94° C for 30 sec, 55° C for 30 sec, 72° C for 1 min, and a final extension step at 72° C for 10 min.

To demonstrate the presence of cellular nucleic acids in the specimens, control primers complementary to cellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA [Hilton et al., 1993] or β-actin mRNA [Ben-Ezra et al., 1991] were used in RT-PCR (Table I). In both cases, the primers were chosen to span an intron, so that PCR products generated for DNA and RNA could be differentiated by size. Times and temperatures for PCR were the same as for enteroviral PCR with the exception that the annealing temperature in GAPDH PCR was 60° C.

Analysis of PCR Products

Ten microliters of the final reaction products were electrophoresed in 2% agarose gels, stained with ethidium bromide (1μg/ml), and visualized under UV illumination. Southern blotting and hybridization [Heino et al., 1994] with a digoxigenin-labeled internal enterovirus-specific probe (Table I) were performed to confirm the specificity of PCR. Moreover, PCR products of correct size were cloned in pGEM-T vector (Promega) and sequenced by the chain-termination method using the (–) and (+) primers and modified T7 DNA polymerase (Sequenase version 2; United States Biochemical Corp., Cleveland, OH) as described earlier [Arola et al., 1996]. Eighty-base pair (bp) long sequences, covering nucleotides 462–542 of the enteroviral genome [Halonen et al., 1995], were selected for sequence analysis. Sequence data were entered manually into a VAX computer using the Genetics Computer Group [GCG; Devereux et al., 1984] Seqed program. The GCG Gap program was used to compare the sequences. Previously published enterovirus sequences used in the comparisons were obtained from the GenBank database.

Preparation of DNA and RNA Probes for In Situ Hybridization

The recombinant CBV3 cDNA clone pCBV3-M1 [Kandolf and Hofschneider, 1985] was digested with *Bam*H I or *Kpn* I restriction endonucleases to generate virus-specific 1.1 kb and 6.2 kb fragments, respectively, corresponding to nucleotides 66–7128 of the viral genome [Klump et al., 1990]. After purification, the cDNA fragments were radiolabeled by random priming using α-^{35}S-dATP and α-^{35}S-dCTP (Amersham, Buckinghamshire, UK) to a specific activity of 3×10^8 cpm/μg of DNA. Single-stranded RNA probes were transcribed from the pCBV3-R1 clone [Hohenadl et al., 1991] using either T7 or SP6 RNA polymerases in the presence of α-^{35}S-UTP to a specific activity of 6×10^8 cpm/μg of RNA. Control DNA and RNA probes were synthesized from nonrecombinant plasmid vectors pBR322 and pSPT18, respectively.

In Situ Hybridization

In situ hybridization was carried out essentially according to the protocol described by Kallajoki et al. [1990] with the exception that 10–15 μg/ml of proteinase K was used in the prehybridization treatment of the specimens. When using RNA probes, nonhybridized single-stranded RNA was digested by RNase A (20 μg/ml) in 10 mM Tris-HCl (pH 8.0), 0.5 M NaCl at 37° C for 15 min. For autoradiography, the slides were treated as described earlier [Kallajoki et al., 1990], exposed at 4° C for 2 to 3 weeks, developed, and counterstained with hematoxylin.

RESULTS

Clinical Presentation

Twenty-nine of the 33 patients (88%) presented with fulminant congestive heart failure. Arrhythmias and/or chest pain were the reasons for cardiac examination in two patients and noncardiac symptoms in another two patients. In 17 patients (51%), the presentation was related to acute infection: 5 patients exhibited symptoms and signs of infection at presentation, whereas 12 patients had a history of infection within 6

months of presentation. Six (50%) of the patients with a recent infection had reported incomplete recovery with nonspecific symptoms, such as tiredness, malaise, lowered physical performance, and orthopnea, progressing towards presentation. One patient had a history of transient heart failure during an acute respiratory tract infection more than 15 years before presentation.

Laboratory Assessment

Virus isolation and subsequent neutralization typing identified CBV4 in a nasopharyngeal specimen from a 3-year-old male patient at diagnosis. As the patient had earlier been at regular cardiologic follow-up because of suspected cardiac disease, enteroviral infection in this case could have triggered latent cardiomyopathy to be clinically manifested but, on the other hand, could also have been an incidental finding. All other virus isolations and antigen detections from various clinical samples, available from 12 patients (36%), were negative. Results of viral serology, carried out using complement fixation or enzyme immunoassay, were available from 22 patients (67%) and paired serum samples from 13 of them. No serological evidence of recent enteroviral infections was obtained. Two patients had fourfold decreases in IgG-titers against parainfluenza virus type 1 in paired serum samples, suggesting infections caused by this virus in the immediate past; however, the role of the virus in the etiology of myocardial disease remained unknown. Results of bacterial serology were available from 10 patients (30%) without diagnostic findings. All bacterial cultures (n = 34) from pharynx, stool, urine, blood, and cerebrospinal fluid were negative. Nine patients (27%) had evidence of mostly mild myocardial injury demonstrated by increased serum LD-1 or CK-MB concentrations, or both. HLA-DR4, earlier reported to be associated with IDCM in 49% of the cases [Carlquist et al., 1991], was identified in three of the eight patients (38%) from whom data were available.

RT-PCR

GAPDH mRNA, producing a 234 bp-long product in PCR [Hilton et al., 1993], could only be amplified from 17 IDCM specimens (27%). Earlier studies have suggested that because of marked degradation of nucleic acids during sample preparation, only short target segments (less than 200 bp) can be amplified successfully from fixed, paraffin-embedded material [Foss et al., 1994; Karlsen et al., 1994]. Therefore, the specimens were also tested using oligonucleotide primers with which a 150 bp-long product of β-actin mRNA can be produced in RT-PCR [Ben-Ezra et al., 1991]. Using these primers, an additional 35 specimens appeared positive by PCR. Thus, cellular nucleic acids could be amplified in 52 (81%) of the 64 myocardial specimens from 32 of the 33 patients. These specimens were considered suitable for subsequent detection of enteroviral RNA.

Oligonucleotide primers for enteroviral PCR were selected from the 5′-untranslated region of the picornaviral genome, which is known to be highly conserved among enteroviruses and rhinoviruses [Rivera et al., 1988]. With the selected primer pair, a wide range of picornaviruses can be detected, including all CBVs, most coxsackie A viruses, echoviruses (except echoviruses 22 and 23), polioviruses, and rhinoviruses [Halonen et al., 1995]. Moreover, our test was sensitive (100 ag of purified CBV3 RNA, corresponding to approximately 20 enteroviral genomes, could be detected) and the length of the amplicon (120 bp) was suitable for formaldehyde-fixed, paraffin-embedded specimens to be investigated [Foss et al., 1994; Karlsen et al., 1994]. Formaldehyde-fixed, paraffin-embedded myocardial specimens obtained 5–10 days postinfection from CBV3-infected BALB/c mice [Arola et al., 1995] were all positive for enteroviral RNA. However, picornaviral RNA was detected in only one patient with IDCM. Interestingly, one control specimen from a 2-month-old female patient with an unknown error of lipid metabolism was also positive by PCR. All other specimens as well as "blank" (water substituted for template) negative controls through all steps from sample preparation to PCR were negative.

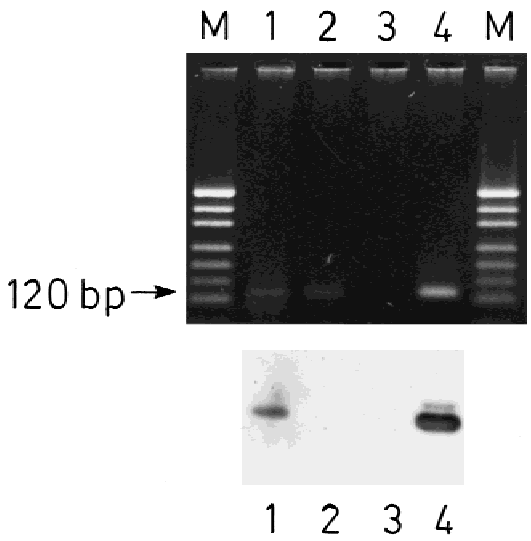

Fig. 1. Detection of picornaviral RNA by RT-PCR in IDCM in the young. Agarose gel electrophoresis (**upper**) demonstrates a 120 bp amplicon corresponding to picornaviral RNA in two myocardial specimens. After Southern hybridization with an enterovirus-specific probe (**lower**), only one of the specimens remains positive. **Lane 1:** EMB specimen from a 19-year-old male patient with IDCM. **Lane 2:** Myocardial autopsy specimen from a 2-month-old female patient with an inborn error of lipid metabolism. **Lane 3:** Negative control (water substituted for template in RT-PCR). **Lane 4:** Positive control (formaldehyde-fixed, paraffin-embedded myocardial specimen from a CBV3-infected BALB/c mouse). M, DNA molecular weight marker.

Southern Hybridization and Sequence Analysis of PCR Products

Southern hybridization [Heino et al., 1994] using a digoxigenin-labeled internal enterovirus-specific probe [Halonen et al., 1995] confirmed the PCR result of the patient with IDCM (Fig. 1). However, the specimen

from the control patient remained negative by hybridization (Fig. 1). Sequence analysis of the 80 bp-long amplified viral segment from the patient with IDCM revealed 100% homology between the viral sequence and echovirus 1 (Fig. 2A), whereas the amplified sequence from the control patient exhibited significant homology (95%) with human rhinovirus 16 (Fig. 2B).

In Situ Hybridization

In situ hybridization with ^{35}S-labeled cDNA and RNA CBV3 probes gave positive signals in CBV3-infected murine myocardial specimens. No enterovirus-positive signals were detected in the specimens from patients with IDCM or from controls.

Case Report

A 19-year-old young man, earlier healthy and without any history of familial myocardial disease, came to a district hospital in January 1991 with fulminant congestive heart failure. He had had a mild upper respiratory tract infection 1 month earlier and during the last 2 weeks he had suffered from progressive orthopnea, abdominal pain, and malaise. On physical examination, sinus tachycardia 120/min, S_3 gallop rhythm, peripheral edema, and liver enlargement (4–5 cm from the right costal margin) were observed. Echocardiographic examination revealed dilatation of the left ventricle (left ventricular end-diastolic dimension 68 mm, 3 SD) and other cardiac chambers, decreased myocardial contractility (FS 10–15%), and secondary mitral and tricuspid regurgitations. Laboratory assessment did not reveal any specific infective etiology underlying the disease. Aggressive medical treatment with angiotensin-converting enzyme inhibitors, digitalis, diuretics, and anticoagulative therapy was started. Cardiac catheterization and EMB (six specimens from the right interventricular septum) were carried out 3 months later; no signs of myocarditis were seen. The disease had progressed despite medication: FS had decreased to 5–10%. Two months later, ventricular arrhythmias appeared and the patient complained of dyspnea even on minor physical exertion. Finally, he underwent a heart transplantation 5 months after initial presentation. Explanted heart showed myocyte hypertrophy, interstitial fibrosis, and patchy endocardial thickening in the left ventricle, but no inflammation with necrosis and/or degeneration of adjacent myocytes indicating myocarditis [Aretz et al., 1986]. By a retrospective PCR analysis, enteroviral RNA could be detected in formaldehyde-fixed, paraffin-embedded EMB specimens, but not in any of the three specimens from the explanted heart. A serum sample taken at the same time as the EMB specimens was negative by enteroviral PCR, thus excluding viremia.

DISCUSSION

Of potential causes of IDCM, the role of enteroviruses, generally considered the most common agents of viral myocarditis in humans [Woodruff, 1980], has been studied extensively. Although the majority of patients with myocarditis make a full recovery, there is a group of patients in whom chronic myocardial disease develops [Billingham and Tazelaar, 1986; Quigley et al., 1987]. However, as enteroviruses typically cause lytic infections in vitro, the possibility that they could cause persistent infections was long ignored. Only upon development of molecular diagnostic tests, based on nucleic acid hybridizations and PCR, was the importance of viral involvement in chronic myocardial disease realized anew [Kandolf et al., 1987; Jin et al., 1990].

Studies detecting enteroviral genomes in adult patients with IDCM have provided variable and inconsistent results [Kandolf et al., 1987; Archard et al., 1988; Bowles et al., 1989; Jin et al., 1990; Weiss et al., 1991; Grasso et al., 1992; Keeling et al., 1992; Severini et al., 1993; Andreoletti et al., 1995, 1996]. The discrepancy can be explained partly by marked variability in the sensitivity and specificity of the tests used as well as by differences in patient materials, diagnostic criteria, and prevalence of enteroviral infections in local populations. As some of these studies have also detected enteroviral RNA in a number of patients with other cardiac conditions and even in normal hearts [Keeling et al., 1992; Weiss et al., 1992; Andreoletti et al., 1995], it is likely that the mere presence of enteroviral RNA in the myocardium cannot be equated with disease. Therefore, it has been speculated that only certain enterovirus types are probably capable of causing chronic myocardial injury [Weiss et al., 1992]; however, no studies supporting this hypothesis have been published to date.

Only a few reports on the detection of enteroviral RNA in childhood myocarditis or IDCM have been published [Hilton et al., 1993; Martin et al., 1994; Shimizu et al., 1995]. Martin et al. [1994] could show specific viral etiology by PCR in 26 of the 38 specimens (68%) from 34 children with suspected acute viral myocarditis. Remarkably, adenovirus ($n = 15$) could be shown in the specimens twice as often as enteroviruses ($n = 8$). The high proportion of virus-positive specimens in the study by Martin et al. [1994] is best explained by the fact that most specimens were fresh frozen and that all specimens, whether from EMB, explanted hearts, or autopsy, were obtained in the acute phase of the disease (within weeks of presentation). Much lower percentages of virus-positive specimens have been reported in studies on childhood myocarditis using autopsy specimens [Hilton et al., 1993; Shimizu et al., 1995].

In the present study, enteroviral RNA was detected in only 1 (3%) of the 32 patients with IDCM. There are several potential explanations for this finding. First, because of the retrospective nature of the study, only formaldehyde-fixed, paraffin-embedded myocardial specimens, most of them from autopsy, could be used. Nevertheless, cellular mRNA (GAPDH, β-actin) could be detected by RT-PCR in all but 12 of the 64 specimens from 32 of the 33 patients studied. Moreover, oligonucleotide primers for enteroviral PCR were designed

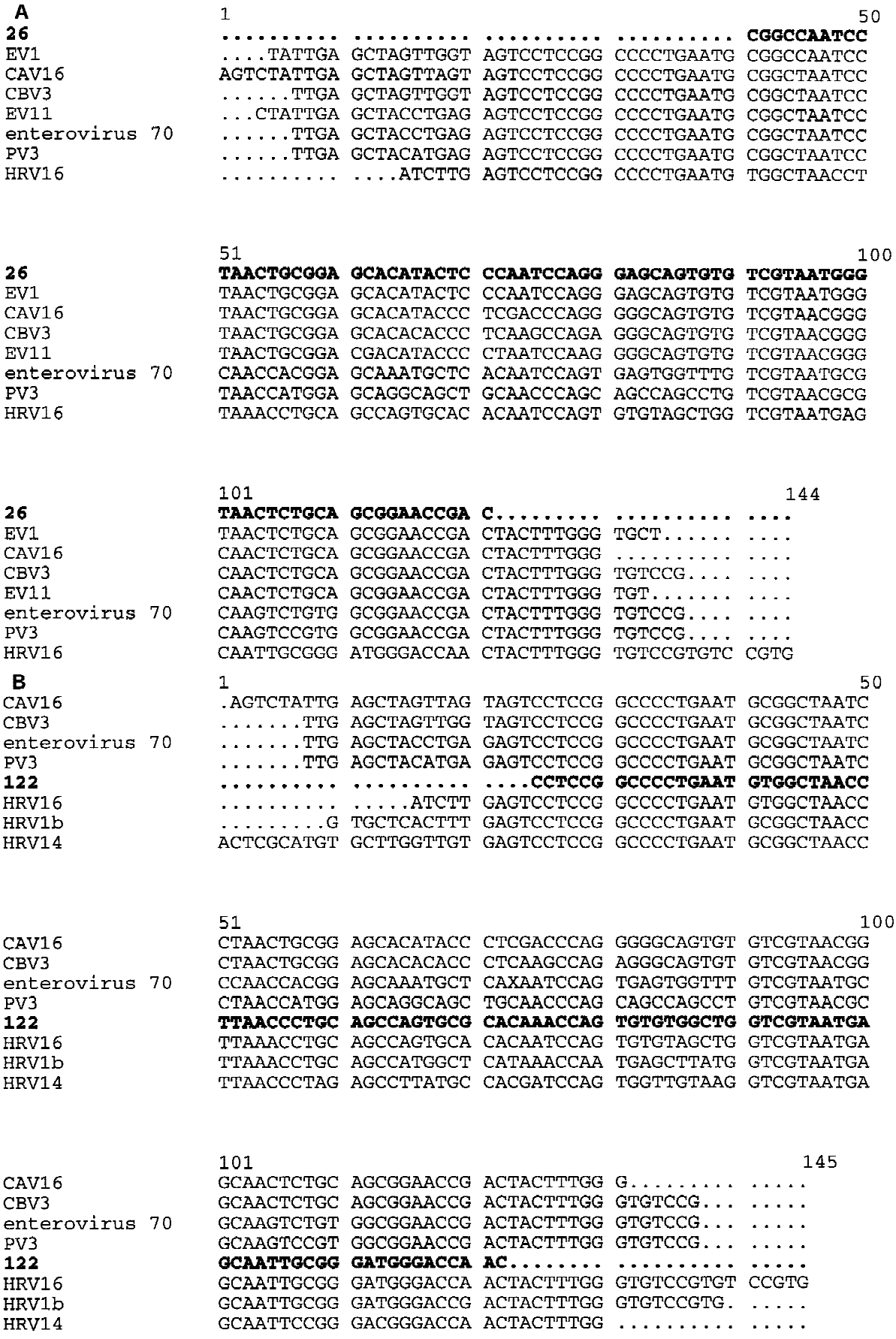

Fig. 2. **A:** Relationship between an 80 bp-long amplified enteroviral sequence from a patient with IDCM (no. 26) and several picornaviral prototypes. Partial sequences obtained from the 5′-untranslated region of the viral genome are compared. **B:** Relationship between the amplified sequence from a control patient (no. 122) and four enteroviral and three rhinoviral prototypes. CAV, coxsackie A virus; EV, echovirus; HRV, human rhinovirus; PV, poliovirus.

to detect virtually all enteroviruses and rhinoviruses and to result in short amplicons, enabling amplification even from nonoptimal biological samples. Second, although the PCR was sensitive, the amount of virus in the myocardial samples might have been too low to be detected. Third, sampling error because of uneven distribution of viral genomes in the tissue [Archard et al., 1988] may lead to underestimation of the frequency of viral RNA in the myocardium. To avoid this, we studied several samples from each patient whenever possible. Fourth, because time from the diagnosis of IDCM to myocardial specimen collection was usually months or even years, viruses that infected initially the myocardium might have been cleared by the immune system during the course of the disease [Dec et al., 1985]. Fifth, IDCM in these patients may have resulted from other infections. Indeed, we could show adenoviral nucleic acids by an earlier described PCR test [Hierholzer et al., 1993] in one patient (data not shown). However, the potential role of herpes simplex virus or cytomegalovirus [Martin et al., 1994], echovirus 22 [Shimizu et al., 1995], and mumps virus [Ni et al., 1997] in the development of IDCM in the studied population remains to be determined. Finally, our patients, representing the most severe end of IDCM in children and adolescents, might have had noninfectious etiology for their disease.

The presence of enteroviral RNA in one adolescent patient with IDCM suggests etiological association between enteroviruses and IDCM. The fact that viral RNA could be detected in the myocardium 3 months after diagnosis but not any longer in the specimens from explanted heart 2 months later can of course be explained by the viral amount being too low to be detected by PCR at the later time point or by an uneven distribution of the virus in the myocardium. However, it is also possible that other mechanisms, e.g., virus-induced autoimmune mechanisms and/or apoptosis [Huber, 1997], were responsible for the progressive nature of the disease. Interestingly, one of the control specimens was also positive for picornaviral RNA by PCR, but remained negative by hybridization; subsequent sequence analysis showed a close relationship between the viral sequence and rhinovirus 16. Because rhinoviruses are generally not considered cardiotropic, the finding is probably best explained by viremia as the patient suffered from a mild respiratory tract infection before death. The finding of one adenovirus-positive IDCM specimen emphasizes the importance of considering also other viruses as potential etiologic agents of IDCM. However, because the adenovirus-positive patient was later diagnosed as having familial cardiomyopathy, the virus mostly precipitated the clinical manifestations of the disease in this case.

In conclusion, the present study shows no significant role for enteroviral persistence in the pathogenesis of childhood IDCM. However, because of the retrospective nature of the present study using only formaldehyde-fixed, paraffin-embedded specimens, our results need to be confirmed in prospective studies using preferably fresh-frozen myocardial specimens. Although enteroviruses are generally considered the most common etiologic agents of viral heart disease, other viruses and noninfectious agents may be more important in the etiology of IDCM and need to be investigated in the future.

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